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Invasion by an exotic tree alters above and belowground ecosystem components

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Abstract With the widespread introduction and invasion of exotic plants there is a need for studies that quantify alterations of basic ecosystem structure and function. Ecosystem invasion by *Melaleuca quinquenervia* significantly altered both above- and belowground ecosystem components in this study. We measured the quantity and nutrient concentration of the litterfall, litter layer, and soil; microbial biomass pools; and rates of potentially mineralizable nitrogen and soil oxygen demand. Annual litterfall was 4.9 times higher in the non-invaded sites and contained 1.9 times more phosphorus than invaded sites. Non-invaded plots contained a larger litter layer compared to invaded plots: 2.4 ± 1.2 kg m^{-2} and 0.62 ± 0.3 kg m^{-2}, respectively. Lower nutrient concentration and quantity of the litter layer in the invaded plots led to changes in the aboveground storage of nutrients. In the invaded plots there was four times less carbon, seven times less nitrogen, and ten times less phosphorus stored in the organic litter layer compared to the non-invaded plots. Microbial biomass nutrient pools were consistently lower at both the 0–5 cm and 5–15 cm depth in the invaded soils compared to non-invaded soils, indicating a plant mediated change. Although *M. quinquenervia* altered microbial community structure, microbial activities were not different between invaded and non-invaded plots at either depth as measured by rates of soil oxygen demand and potentially mineralizable nitrogen. These changes may affect both native plant growth and water quality, and may act to promote and maintain site dominance by *M. quinquenervia*.

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Introduction

Ecosystem invasion by exotic plant species poses a significant threat to community biodiversity, function, and stability (Mack and D'Antonio 1998). Exotic plants often thrive in new habitats when they leave their natural enemies behind and subsequently

experience less top-down regulation from herbivory (D'Antonio and Meyerson 2002). In addition, bottom up forces like high or low resource availability may facilitate colonization and allow exotics to out-compete natives and dominate ecosystems (Blumenthal 2006). For example, the invasive plant *Myrica faya*, a nitrogen-fixing tree native to the Canary Islands, invaded Hawaii and increased the supply of soil nitrogen by 90 folds (Vitousek and Walker 1989).

Species alteration may reduce or eliminate an ecosystem's ability to provide ecological goods and services, such as waste processing and carbon sequestration (Fenn et al. 2003). Functional changes in plant structure and growth rate can alter above- and belowground nutrient pool sizes (Ehrenfeld 2003; Jackson et al. 2002). Although this issue is of global concern, the invasion of exotic plants has been a significant problem in the state of Florida, especially South Florida, which is home to Everglades. The Everglades is an extensive forest and graminoid wetland community that once occupied 4,000 square miles of the state's lower-peninsula, over twice its present day land area (Gunderson 1994). This greatly altered ecosystem has proved an ideal habitat for many exotic plant species.

One of the most problematic species in this ecosystem is *Melaleuca quinquenervia* (Cav.) Blake otherwise known as the paper-bark tree, cajeput, punk tree, or white bottlebrush tree (hereafter referred to as *M. quinquenervia*). *M. quinquenervia* is a member of the Myrtacaea family, sub family Leptospermoidae. This evergreen tree grows up to 30 m in height and its home range includes tropical wetland areas along the eastern coast of Australia (Kaufman and Smouse 2001). It was introduced into southern Florida in 1886 (Dray et al. 2006), originally for sale as an ornamental, but later was used for erosion control, as a forestry crop, and as an agricultural windrow plant (Bodel et al. 1994; Meskimen 1962; Stocker et al. 1981). *M. quinquenervia* has readily invaded and dominated most natural areas of South Florida, including bayhead tree islands, sawgrass prairies, pine flatwoods, pastures, and cypress forests (Bodel et al. 1994).

M. quinquenervia has several morphological adaptations that make it well suited to the dynamic environmental condition of Florida including tolerance to shifts in temperature and pH, moderate salinity, and variable water regimes (Kaufman and

Smouse 2001). Once established, *M. quinquenervia* trees have a high growth rate and reach reproductive maturity at 3 years after which it may flower two to five times a year (Bodel et al. 1994). A single 21 m tall *M. quinquenervia* tree can hold an estimated 9 million seeds in its canopy which, once released, may remain viable in the soil for up to 3 years (Rayamajhi et al. 2002; Van et al. 1998). Seeds are held in capsules that open in response to desiccation, frost, or fire (Serbesoff-King 2003). In addition, high concentrations of essential oils found in mature *M. quinquenervia* trees fuel canopy fires that can kill native vegetation. Following destructive canopy fires, *M. quinquenervia* will release massive amounts of seeds, which result in virtual *M. quinquenervia* monocultures (Serbesoff-King 2003).

By the early 1980s it became clear that *M. quinquenervia* was significantly altering the plant communities of the Florida Everglades when a survey completed by the US Forestry Service revealed that 470,000 acres of land was covered by "pure" *M. quinquenervia* stands (Bodel et al. 1994). LaRoche and Ferriter (1992) estimated that once *M. quinquenervia* populations reached a critical ecosystem concentration of 2–5%, 95% infestation occurred within 25 years. Once an ecosystem is invaded by *M. quinquenervia*, native plant communities are significantly altered and wildlife diversity and abundance have been found to decrease (DiStefano and Fisher 1983; Mazzotti et al. 1981, 1997).

Although much is known of the aboveground alterations caused by *M. quinquenervia* invasion, virtually nothing is known about the consequences for belowground processes. Plant induced belowground alterations may allow invasives to promote and maintain community dominance and may affect native plant growth and soil and water quality (Ehrenfeld 2003). Elucidation of the extent, duration, and long-term impact of belowground ecosystem alterations following invasions by exotics will help in developing more effective restoration and management techniques.

The major objective of this work was to determine if invasion of the exotic plant *M. quinquenervia* induced both above- and belowground changes in a *Taxodium distichum* (L.) L.C. Rich var. *nutans* (Ait.) Sweet dominated eco-tone forest. In particular, we were interested in the consequences for nutrient pools and microbial population dynamics. Five hypotheses were

tested: (1) *M. quinquenervia* invasion will alter the quantity of above- and belowground biomass compared to *T. distichum*; (2) *M. quinquenervia* invasion will lower the quantity and nutrient concentration of litterfall and litter layer; (3) litter decomposition rates will be lower in invaded plots; (4) microbial nutrient pools will be smaller in the invaded soils; and (5) microbial activity will be lower in invaded soils.

Materials and methods

Site description

The study site was located in the Belle Meade Tract of the Picayune Strand State Forest in Collier County, Florida. This area consists of nearly level, poorly drained, low fertility soils which are loamy, siliceous, hyperthermic Arenic Glassoqualfs. The soil series is Pineda–Boca–Hallandale which is characterized by moderately to poorly drained sands over-lying lime-stone bedrock at a depth of approximately 1.4 m (USDA 1998). The water table fluctuates annually between greater than 15 cm below the soil surface to approximately 25 cm above. The area has a distinct wet season from approximately July–December and a dry season from January–June. Average annual rainfall in this region is approximately 1.36 m (SERC 2007).

Vegetation in this area was a mixed *Pinus elliottii* Englem-*T. distichum* forest with hardwood under-story and a few mature *M. quinquenervia* trees. In August 1998, the area experienced a canopy fire that killed many of the native trees and precipitated a large release of seeds from *M. quinquenervia* trees. This was followed by a concomitant pulse of recruitment of *M. quinquenervia* seedlings that exceeded densities of 100 plants per square meter. The burned area was thus transformed into a landscape of sparsely distributed mature *M. quinquenervia* trees surrounded by a virtual monoculture of even-aged saplings. Those areas not burned maintained their original structure and tree species composition (Tipping, unpublished data).

Vegetation sampling

In February 2005, seven plots were established in each of two areas: an area dominated by mature *M. quinquenervia* trees and saplings (hereafter referred to as "invaded plots"), and an area dominated by mature

T. distichum trees (hereafter referred to as "non-invaded plots"). Each circular plot measured 6 m in diameter and contained a mature tree in the center. We estimated standing biomass in the non-invaded plots using *T. distichum* only since it comprised $77 \pm 8.3\%$ of the woody species present. Aboveground biomass of the *T. distichum* was estimated from three separate regression equations using diameter at breast height (DBH) and total tree height (HT) (Mitsch and Ewel 1979). DBH of all *T. distichum* trees within the plot was measured with a diameter tape. The height of larger trees was estimated with a clinometer while shorter trees were measured directly with a tape measure. The general form of the *T. distichum* biomass regression equation used was: $y = a \times x^b$; where $y = $ biomass, and $x = (dbh \times ht)^{-1/2}$.

Aboveground biomass of mature *M. quinquenervia* trees was estimated using measurements of diameter outside bark (DOB) (Van et al. 2000). The regression equation used was: $\log e (W) = -1.83 + 2.01 \times \log e (DOB)$ (where $W = $ total biomass). The bio-mass of the *M. quinquenervia* under-story in each plot was measured directly by collecting all of the standing vegetation in two 1 m^2 sub-plots. All biomass measurements were reported on a dry weight basis.

To determine litterfall in each plot, four 0.25 m diameter litter traps were placed at four cardinal directions approximately 1 m from the base of the mature tree. Litterfall was collected every 60 days for 1 year (nutrient analysis was completed for the first 8 months of litterfall sampling). The organic litter pool in each treatment was sampled from two 0.1 m^2 sub-plots. The litter layer was separated into un-decomposed (Oi), moderately decomposed (Oe), and humified (Oa) layers. Litterfall and the litter pool samples were air dried and reported on a dry weight basis. Belowground biomass in each plot was estimated using two-5 cm diameter soil cores which were taken at a distance of 1 m from the base of the mature tree. Each belowground biomass core was separated at two depths, 0–5 cm and 5–15 cm and sieved to separate fine roots which were then rinsed, air dried, and weighed.

Soil sampling and analyses

In February 2005, four-5 cm diameter soil cores were taken in each plot at a distance of 1 m from the base of the native or invasive mature tree. Each core was

separated at two depths, 0–5 cm and 5–15 cm and combined to form two composite soil samples per plot at each depth. Soil samples were returned to the laboratory, sieved to remove roots and large plant debris, homogenized, and kept at 4°C for a maximum of 10 days before microbial analysis. Percent moisture and bulk density of soils were determined by drying 20–30 g sub-samples of field-moist, sieved, and homogenized soil at 70°C for 3 days. Bulk density and percent moisture were determined on a wet soil weight basis and pH was measured on a 2:1 water:soil slurry with an Accumet Research, AR50 dual channel pH/ion/conductivity meter. Soil organic matter was measured by loss on ignition from 0.2 to 0.5 g samples of dried and ground soils, which were first measured into 50 ml beakers (Luczak et al. 1997).

Nutrient analyses

Dried and ground soil and plant material was analyzed for percent carbon (C) and nitrogen (N) on a Thermo-Electron, 1112 Series elemental analyzer. Total phosphorus (P) was determined by a two-phase acid extraction after loss on ignition and measured with an automated ascorbic acid method on a Bran and Luebbe Auto Analyzer 3, Digital Colorimeter (Andersen 1976), Method 365.4 (USEPA 1993).

Microbial population analyses

Microbial biomass carbon (MBC) and nitrogen (MBN) were measured by a chloroform-fumigation extraction method modified from Vance et al. (1987). Two replicates of 1 g field-moist soil samples were weighed into 50 ml centrifuge tubes. One of the replicates was extracted immediately with 25 ml of 0.5 M K_2SO_4, shaken for 1 h, and then filtered through a Whatman #41 filter. The second replicate of soil underwent chloroform fumigation where 0.5 ml of chloroform was added to each tube and the tubes were placed in a dessicator with a beaker containing 30 ml chloroform and boiling chips. The dessicator was then vacuum-sealed for 24 h. After fumigation, the dessicator was alternatively filled with air and evacuated ten times to remove all residual chloroform. The tubes were then extracted as described above and all extracts were stored at 4°C

until analysis for MBC. This was calculated as the difference between the total carbon in the fumigated and unfumigated soil extracts as measured on a Shimadzu TOC 5050C, total organic carbon analyzer. An extraction coefficient of 0.45 was applied to all calculations of microbial biomass carbon (Chen et al. 2005).

To measure MBN, 10 ml of the 0.5 M K_2SO_4 extract from the fumigated and unfumigated tubes, 2 ml of concentrated H_2SO_4, and ca. 0.6 g of Kjeldahl salt catalyst were added to 40 ml digestion tubes. Tubes were placed on a digestion block and brought to 125°C for 2–3 h, then 150°C for 2–3 h until all water was removed, and finally at 380°C for 2–3 h until samples were clear and digestion was complete. Deionized water was added to the acid digestates to reach a final volume of 20 ml. MBN was calculated as the difference between the total Kjeldahl nitrogen in the fumigated and unfumigated acid digestates as measured on a Bran and Luebbe Auto Analyzer, Digital Colorimeter (Method 351.2; USEPA 1993). An extraction coefficient of 0.2 was applied to all calculations for microbial biomass nitrogen (Chen et al. 2005).

Microbial biomass phosphorus (MBP) was measured with the same chloroform-fumigation extraction method described above with the exception that after fumigation, 25 ml of pH adjusted 0.5 M $NaHCO_3$ was used to extract the soil, tubes were shaken for 16 h and extracts were filtered through a 0.45 μm filter (Brookes et al. 1982). Five ml of the soil extracts were digested with 1 ml of 11 N H_2SO_4 and one scoop potassium persulfate (ca. 0.6 g) using the same digestion block program described above. Deionized water was added to reach a final volume of 10 ml. MBP was calculated as the difference between the total phosphorus in fumigated and unfumigated acid digestates and measured with an automated ascorbic acid method on a Bran and Luebbe Auto Analyzer 3, Digital Colorimeter (Method 365.4; USEPA 1993).

Soil oxygen demand (SOD) is a measure of the rate of oxygen consumption during chemical and microbial mediated oxidation during a 24 h dark incubation (Malecki et al. 2004; Reddy et al. 1980). About 5–6 g of field-moist soil were weighed into dark, biological oxygen demand bottles with a magnetic stirrer. Bottles were then completely filled with oxygen-saturated water, put on a stir plate and

agitated for 30 min, and an initial measurement of dissolved oxygen was taken with an Accumet Research, AR40 dissolved oxygen meter and recorded after which bottles were incubated at room temperature for 24 h. After incubation the bottles were again agitated and a final dissolved oxygen reading was taken. Oxygen demand was reported as the difference between the final and initial dissolved oxygen concentrations per gram of soil per hour.

Potentially mineralizable nitrogen (PMN), a biological index, is a measurement of the soil organic nitrogen that can be decomposed and made available for plant uptake. PMN was measured with a method modified from White and Reddy (2000). This method calculates a mineralization potential based on the net production of ammonium during 10 days incubation. In order to determine the initial amount of ammonium in the soil, a set of 1 g field-moist soil samples were weighed into 50 ml centrifuge tubes. The tubes received 25 ml of 0.5 M K_2SO_4 and shaken on a longitudinal shaker for 1 h, and extracts were filtered through a #41 Whatman filter. An additional set of 1 g field-moist soil samples were weighed into 50 ml serum bottles with 5 ml of deionized water. Bottles were sealed with butyl rubber stoppers and aluminum crimps. The bottle head-space was purged with O_2-free N_2 gas for 2–5 min. Bottles were incubated in the dark at 40°C for 10 days then extracted as described above with 25 ml of 0.5 M K_2SO_4 to determine final ammonium concentration. Total ammonium was determined with an automated colorimetric method on a Bran and Luebbe Auto Analyzer 3, Digital Colorimeter (Method 353.2; USEPA 1993).

Statistical analyses

The experimental unit in this study was the mature tree at the center of each plot. Values of measured soil and vegetation characteristics, as well as microbial population size and activity were calculated as a mean for each plot. Student's t tests were used to detect any differences between the measured parameters. Differences are reported as significant for tests with P-values ≤ 0.05. Data that varied from normal distributions were analyzed non-parametrically with the Wilcoxon test. The non-normal datasets are: pH, % moisture, C content (Oi/Oe and Oa), N content (Oi/Oe and Oa), P content (soil 0–5 and 5–15 cm), SOD (5–15 cm), and litterpool biomass (Oi/Oe and total). All statistical analyses were preformed using JMP 7.0.1 software (SAS Institute, North Carolina, USA).

Results

Values and significant differences of soil moisture, pH, bulk density and organic matter content of the soil samples are reported in Table 1. There were no significant differences in the amounts of C and N concentrations in the litterfall between sites (Table 2). However, total P concentrations in the invaded plots averaged 47% less than in the non-invaded plot litterfall (Table 2). There was a significant decrease in C, N and P concentrations of the invaded plot Oi/Oe layer relative to non-invaded plots (Table 2). The Oi/Oe layer in the invaded plots had 31% less total N concentration and 39% less total

Table 1 Mean (+SD) of soil variables measured in the soil of the invaded and non-invaded sites

Variable	Sample location	Non-invaded	Invaded	P
pH	0–5 cm depth	5.4 ± 0.2	5.3 ± 0.3	0.7*
	5–15 cm depth	5.3 ± 0.2	5.0 ± 0.3	0.03*
Moisture (%)	0–5 cm depth	8.2 ± 5.5	4.8 ± 0.2	0.4*
	5–15 cm depth	6.3 ± 1.4	4.1 ± 0.6	0.002*
Bulk density (mg cm^{-3})	0–5 cm depth	1.3 ± 0.2	1.1 ± 0.1	0.04
	5–15 cm depth	1.5 ± 0.1	1.5 ± 0.1	0.85
Organic matter (%)	0–5 cm depth	4.1 ± 2.3	3.9 ± 0.4	0.84
	5–15 cm depth	1.7 ± 0.8	1.3 ± 0.3	0.24

* Indicates non-parametric test

Table 2 Mean (+SD) of carbon, nitrogen, and phosphorus content measured in the litter and soil of the invaded and non-invaded sites

Variable	Sample location	Non-invaded	Invaded	P
Carbon (g g^{-1})	Litterfall	0.5 ± 0.01	0.5 ± 0.01	0.66
	Litter (Oi and Oe)	0.4 ± 0.05	0.5 ± 0.02	0.01*
	Litter (Oa)	0.4 ± 0.04	0.4 ± 0.05	0.6*
Carbon (mg g^{-1})	0–5 cm	14.6 ± 6.7	18.6 ± 3.7	0.2
	5–15 cm	6.5 ± 3.8	4.2 ± 2.0	0.2
Nitrogen (mg g^{-1})	Litterfall	10.2 ± 0.8	8.9 ± 1.4	0.07
	Litter (Oi and Oe)	17.5 ± 2.3	12.1 ± 1.1	0.005*
	Litter (Oa)	20.8 ± 2.0	15.0 ± 1.4	0.005*
	0–5 cm	0.90 ± 0.2	0.88 ± 0.07	0.5
	5–15 cm	0.65 ± 0.25	0.39 ± 0.08	0.04
Phosphorus (µg g^{-1})	Litterfall	220.5 ± 61.6	117.2 ± 15.5	0.008
	Litter (Oi and Oe)	255.4 ± 29.6	156.5 ± 27.8	0.0001
	Litter (Oa)	346.3 ± 50.6	225.8 ± 65	0.03
	0–5 cm	24.4 ± 15.2	18.3 ± 3	0.89*
	5–15 cm	12.4 ± 7.3	6.7 ± 1.2	0.03*

* Indicates non-parametric test

P concentration (Table 2). Nutrient concentrations of the Oa layer in the invaded plots followed the same pattern with a 28% decrease in total N concentration and a 35% decrease in total P concentration compared to the non-invaded plots (Table 2).

There were no significant differences in the rates of SOD or PMN in plots at either depth (Table 3). However, there was a 62% and 72% decrease in mineralization capacity in the subsurface soil of the invaded and non-invaded plots, respectively, and a 71% and 61% decrease in oxygen demand in the subsurface soil of the invaded and non-invaded plots, respectively.
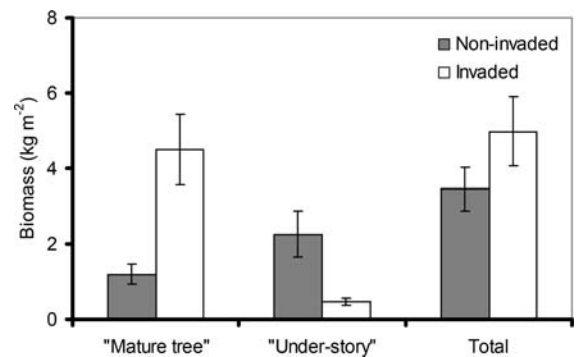
The non-invaded plant community was a closed-canopy mature *P. elliottii–T. distichum* forest. On

Table 3 Mean (±SD) of microbial activity from different soil depths in the invaded and non-invaded sites

Variable	Sample location	Non-invaded	Invaded	P
Potentially mineralizable nitrogen (µg g^{-1} day^{-1})	0–5 cm	1.04 ± 0.4	1.3 ± 0.3	0.3
	5–15 cm	0.3 ± 0.2	0.5 ± 0.3	0.1
Soil oxygen demand (µg g^{-1} day^{-1})	0–5 cm	2.5 ± 1.2	2.4 ± 0.5	0.6
	5–15 cm	0.97 ± 0.4	0.7 ± 0.2	0.13*

* Indicates non-parametric test

average, the "Mature tree" in the non-invaded plots composed 35% of the total standing biomass (Fig. 1). In addition, the "Understory" of each non-invaded plot contained six additional mature *T. distichum* trees that contributed another 64.8% of the total biomass and seven *T. distichum* saplings that comprised the remaining 0.2%. The community structure in the invaded plots consisted of sparse mature *M. quinquenervia* trees with an under-story of dense and even-aged saplings. On average the "Mature tree" in each plot contributed 90% of the total biomass. The "Understory" was comprised of

Fig. 1 Mean standing biomass (±SE) of the "Mature trees", "Under-story" vegetation, and total biomass for the invaded and non-invaded sites

1131 *M. quinquenervia* saplings that contributed the remaining 10% of the total biomass (Fig. 1).

The mean density ($\pm$SE) of *M. quinquenervia* saplings in the invaded plots was 40 ± 23 saplings m^{-2} while a mean of 16 ± 2 *T. distichum* trees was present in un-invaded plots. The mean height of the *T. distichum* and *M. quinquenervia* "mature" trees was 8.27 ± 0.35 m and 10.67 ± 0.47 m, respectively. The mean DBH of the *T. distichum* and *M. quinquenervia* "mature" trees was 17.89 ± 1.75 cm and 30.47 ± 4.7 cm, respectively. There was no significant difference in total aboveground biomass between the invaded and the non-invaded plots (Fig. 1, $P = 0.07$). We estimated the average aboveground biomass of *T. distichum* in non-invaded plots to be 1.2 ± 0.3 kg m^{-2} for the "mature tree" and 2.26 ± 0.6 kg m^{-2} for all other under-story trees, with a total of 3.46 ± 0.6 kg m^{-2} of *T. distichum* biomass (Fig. 1). *M. quinquenervia* aboveground biomass in the invaded plots was 4.5 ± 0.9 kg m^{-2} for "mature" trees and 0.48 ± 0.1 kg m^{-2} for understory *M. quinquenervia*, with an overall biomass total of 4.98 ± 0.9 kg m^{-2} (Fig. 1).

Root biomass was higher in the surface (0–5 cm) soil layer of invaded plots compared to the non-invaded plots, of 196.2 ± 26.2 g m^{-2} ($\pm$ SE) and 130.5 ± 23.5 g m^{-2}, respectively ($P = 0.09$). In addition, root biomass was significantly higher in the subsurface (5–15 cm) soil layer of invaded plots compared to the non-invaded plots, with means ($+$SE) of 391.6 ± 40.7 m^{-2} and 131.4 ± 12.4 g m^{-2}, respectively ($P = 0.0005$). Total root biomass was significantly higher in the invaded plots compared to the non-invaded plots 587.8 ± 42.4 g m^{-2} and 261.9 ± 31.6 g m^{-2}, respectively ($P < 0.0001$).

Average litterfall was significantly higher 52.2 ± 4.3 g m^{-2} yr^{-1} in the invaded plots and 257.5 ± 40.1 g m^{-2} yr^{-1} in the non-invaded plots, a 4.9 fold difference ($P = 0.0021$). There was a temporal component to litterfall differences among the treatments which existed from July 2005 through October 2005 (Fig. 2). Overall, litterfall production per unit of tree biomass was approximately 6.6 times greater in the non-invaded plots compared to the invaded plots: 88.2 ± 16.5 g kg^{-1} and 14.5 ± 2.9 g kg^{-1}, respectively ($P = 0.0061$).

The non-invaded plots had a significantly larger total litter pool compared to the invaded plots (Fig. 3, $P = 0.007$). The Oi/Oe layer was three times larger
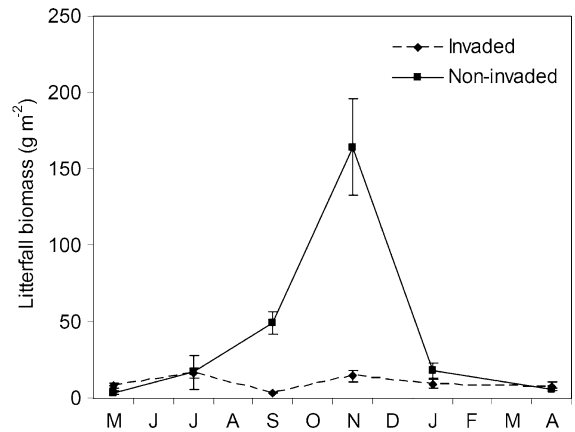

Fig. 2 Mean litterfall biomass ($\pm$SE) of each sample collection for the invaded and non-invaded sites
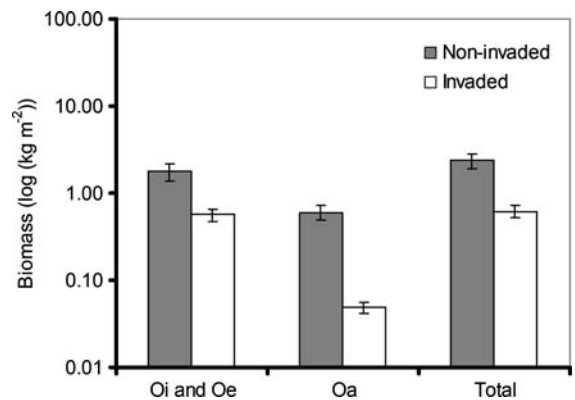

Fig. 3 Mean litter pool biomass ($\pm$SD) of the Oi and Oe, Oa, and total litter pools for the invaded and non-invaded sites

in the non-invaded plots compared to the invaded plots, with values of 1.79 ± 0.11 kg m^{-2} and 0.57 ± 0.25 kg m^{-2}, respectively ($P = 0.03$). The Oa layer was 12 times larger in the non-invaded plots compared to the invaded plots, with mean values of 0.6 ± 0.03 kg m^{-2} and 0.05 ± 0.02 kg m^{-2}, respectively ($P = 0.002$). The turnover time of the total litter pools was not different between plots with a mean ($\pm$SE) of 10.3 ± 2.3 and 11 ± 1.9 years in the non-invaded and invaded plots, respectively ($P = 0.3$).

MBC, MBN, and MBP were all lower in the invaded plot soils at both soil depths (Fig. 4). MBC was 17% lower in the invaded soils for the 0–5 cm soil depth and 25% lower for the 5–15 cm depth (Fig. 4a, $P = 0.08$ and 0.07) with MBN and MBP following a similar pattern. MBN was 34% lower in
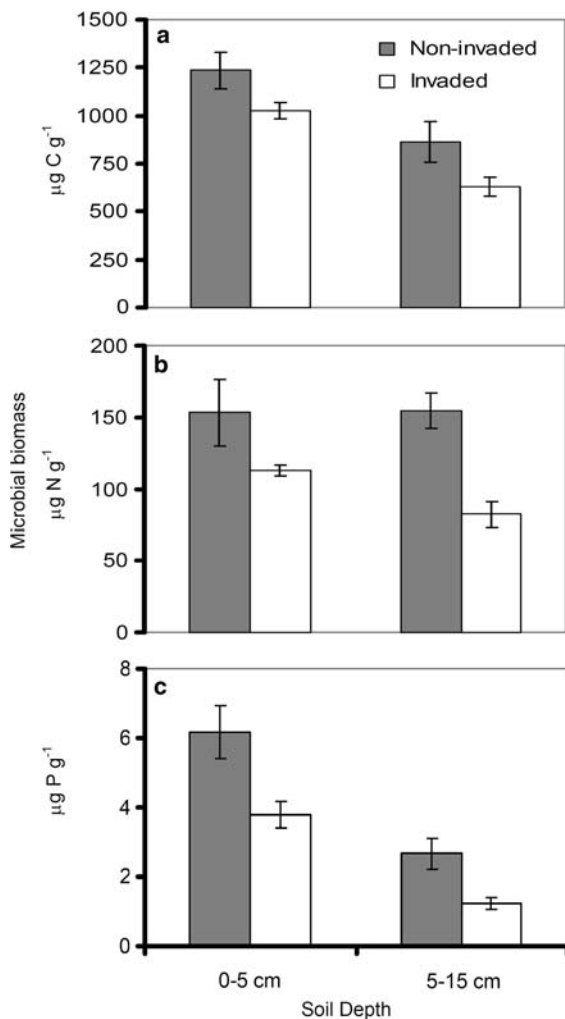
Fig. 4 Mean microbial biomass carbon (**a**), nitrogen (**b**), and phosphorus (**c**) (±SE) of 0–5 cm and 5–15 cm soil depths for the invaded and non-invaded sites

the invaded soils for the 0–5 cm soil depth and 48% lower for the 5–15 cm depth (Fig. 4b, $P = 0.13$ and 0.0002). MBP was 39% lower in the invaded soils for the 0–5 cm soil depth and 55% lower for the 5–15 cm depth (Fig. 4c, $P = 0.02$ and 0.01).

Discussion

Species invasion and aboveground effects

Invasion of a *P. elliottii*–*T. distichum* eco-tone by *M. quinquenervia* resulted in changes in both forest structure and ecosystem nutrient pools. The lower

litterfall in invaded plots may be explained in part by differences in plant characteristics. *T. distichum* is a deciduous tree that sheds all of its leaves in late fall to early winter (Ewel and Odum 1984). In this study there was a distinct seasonality to the litterfall in the non-invaded plots; 83% of the litterfall was produced from August to November (Fig. 2). Litterfall measured in the non-invaded plots was similar to values reported by Brown (1981).

In contrast, *M. quinquenervia* is an evergreen tree which holds its leaves in the canopy for at least 2 years (Van et al. 2002). Unlike the non-invaded plots, litterfall in the invaded plots was lower than reported in previous studies (Greenway 1994; Van et al. 2002). Differences among ours and previous studies may be explained by differences in *M. quinquenervia* forest structure. Both of the aforementioned studies were conducted in closed-canopy *M. quinquenervia* stands with densities of mature trees (DBH > 20 cm) of approximately 0.1 and 0.15 trees m^{-2}, respectively. The *M. quinquenervia* forest in our study was in the early stages of forest development with only 0.04 mature trees m^{-2}.

Another factor potentially influencing litterfall in this study was the presence of two biological control agents released on *M. quinquenervia* over the past decade: *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) and *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae). Tipping et al. (2008) found that herbivory from *O. vitiosa* significantly altered *M. quinquenervia* growth and reproduction. Although not quantified, the *M. quinquenervia* leaves collected during litterfall sampling in this study had noticeable insect feeding damage. High levels of herbivory could explain the low litter production per unit standing biomass in the invaded site.

Both the quantity and nutrient concentration of the litterfall in the invaded plots were lower compared to the non-invaded plots (Table 2). The total litterfall in the invaded plots had 47% less total phosphorus concentration compared to the non-invaded plots and therefore approximately eight times more phosphorus per m^{-2} was added to the forest floor in the non-invaded area during the course of this study. The nutrient poor, sandy surface soils of these forests do not have a large capacity to store nutrients vital for ecosystem maintenance. Therefore, reducing the quantity of nutrients returned to the soil through litterfall could have significant consequences for forest

productivity. This finding may represent a potential mechanism through which *M. quinquenervia* is able to promote and maintain dominance by reducing overall ecosystem resources for competing plant species.

The litter pool was also significantly larger in the non-invaded plots with almost four times as much litter accumulated on the forest floor compared to the invaded plots (Fig. 3). There was also a significant decrease in the nutrient concentration of the litter pool (Table 2). Lower nutrient concentration and quantity of the litter layer in the invaded plots led to changes in the aboveground storage of nutrients where four times less carbon, seven times less nitrogen, and ten times less phosphorus were stored in the organic litter layer per m^{-2} compared to the non-invaded plot. Reductions in aboveground nutrient inputs and storage caused by invasion may have significant consequences for long-term ecosystem function.

We hypothesized that the lower nutrient concentration in the *M. quinquenervia* litter would decrease its turnover time in the invaded plots by assuming that the ecosystem is in steady state (Chapin et al. 2002). However, despite differences in nutrient concentration, turnover time of the litter pools was not different between the two plots indicating that other environmental factors such as organic carbon liability may be controlling the rate of litter decomposition. This lack of difference in turnover time, coupled with differences in litterfall production, explains the larger litter layer present in the non-invaded plots.

Although no direct measurements of *M. quinquenervia* decomposition have been published, we reanalyzed litterfall and litter accumulation data from Greenway (1994) to obtain an estimate of ca. 1,560 days or 4.3 years for the turnover time of *M. quinquenervia* litter. This is significantly faster than the 11 ± 1.9 year turnover estimate in our study. A similar pattern was seen in measurements of turnover time for the *T. distichum* litter. Nessel and Bayley (1984) estimated 3 years for the turnover time of *T. distichum* litter compared to our estimate of 10.3 ± 2.3 years. The reasons for these differences are unknown but our study site was subjected to multiple high wind events caused by the close passage of several hurricanes, which likely increased estimates of the litter pool by increasing litterfall rates. Also we sampled the litter pool a month after

the *T. distichum* leaf-drop and before the period of seasonal inundation. Repeated sampling throughout the year, to quantify seasonal variation in the litter pool, would provide a more accurate estimation of turnover time.

Species invasion and belowground effects

A potential mechanism whereby *M. quinquenervia* out-competes native species is through a highly competitive root system. Lopez-Zamora et al. (2004) found that *M. quinquenervia* plants developed significantly higher root densities than native grass competitors and maintained high root densities throughout the soil profile independent of moisture content. DiStefano and Fisher (1983) found that *M. quinquenervia* stands had higher root densities in the upper 20 cm of soil when compared to stands of both *Pinus elliottii* and *Eucalyptus gradis.* Our study supports these findings: total fine root densities were significantly higher in the invaded plots. In addition, the ratio of aboveground to belowground biomass was higher in non-invaded plots; 13:1 vs. 9:1 in invaded plots ($P = 0.13$). The relatively higher per tree root biomass of *M. quinquenervia* may result in greater capture of nutrient resources than native species.

MBC, MBN, and MBP were consistently lower in the invaded plots (Fig. 4). Although the mechanisms by which *M. quinquenervia* alters the soil environment were not explored in this study, differences in microbial pools between plots suggest a plant influence. *Melaleuca alternafolia*, a close relative of *M. quinquenervia*, produces volatile essential oils with antimicrobial properties (Carson et al. 2006). DiStefano and Fisher (1983) found that extracts of *M. quinquenervia* leaves reduced seed germination and seedling growth of native plant species. In addition, fungal infection of plant embryos was significantly reduced by a *M. quinquenervia* leachate treatment. Therefore, allelopathic compounds produced by *M. quinquenervia* may be responsible for alterations in microbial biomass pools but further study is needed.

Although a gross measurement, alteration of microbial biomass nutrient concentrations may suggest shifts in microbial community composition. On average soil bacteria have C:N ratios of 4:1–10:1, while fungi range from 8:1 to 15:1 (Davet 2004). In this study, the C:N ratio of the microbial biomass

was 11:1 and 9:1 in the surface soil and 9:1 and 7:1 in the subsurface soil of the invaded and non-invaded plots, respectively. Although it is expected that fungal biomass would dominate in the acidic soils of these forests, plant mediated alteration of the rhizosphere may favor one species over another. Alteration of native microbial community composition may further decrease competition from native plants and therefore support *M. quinquenervia* dominance. Studies have shown that through a series of aboveground-belowground feedback loops, plant community structure can drive changes in soil community size and composition (Batten et al. 2006; Wardle et al. 2004). Thus, the loss of the associated mycorrhizal associations could decrease the competitive ability of the native plant community.

Changes in microbial species composition may alter turnover rates of soil nutrient pools, which in turn may change rates of ecosystem nutrient cycling. For example, if fungal biomass were to become more prevalent in the invaded plots, then substrate use efficiency could be greater thereby increasing the availability of carbon substrates (Davet 2004). In addition, microbial communities with higher C:N biomass ratios have lower requirements for nitrogen and will therefore mineralize nitrogen at lower concentrations (Eviner and Chapin 2003). In the low fertility soils of southern Florida, this could alter the availability of nutrients necessary for ecosystem maintenance.

Microbial biomass accounted for a large proportion of the total nutrient storage in surface soils of the plots. For example, in the 5–15 cm soil depth, MBP accounted for 23% of the total phosphorus in the non-invaded and 19% in the invaded sites. Therefore, any reduction in the size of the microbial pools could increase nutrient mobility and result in leaching losses. Vitousek and Matson (1984) found that microbial immobilization of nitrogen was the primary control of nitrogen retention in forest soils after an ecosystem disturbance. Soils in the invaded plots will stabilize approximately 76 g less phosphorus by microbial immobilization than non-invaded plots. This is over 53 times as much phosphorus as is added to the same area by litterfall each year. Reduced nutrient storage means increased nutrient leaching, with significant consequences for down-stream aquatic communities.

Although the size of the microbial pool was significantly lower in the invaded plots, microbial activity, as measured by soil oxygen demand, was not different between plots. Malecki et al. (2004) reported that a seasonal decrease in MBP corresponded with a slower rate of oxygen consumption in a wetland. Our study did not support this finding (Table 3). Microbial biomass was consistently lower in soils in invaded plots, however, there was no difference in the rate of oxygen consumption between sites at either depth.

It was hypothesized that the rate of soil nitrogen supply as measured by PMN would be lower in invaded soils. White and Reddy (1999) reported a positive correlation between the rates of PMN and microbial biomass pools. In addition, Kourtev et al. (2003) found that the experimental introduction of two exotic plants significantly altered rates of PMN in a New Jersey forest soil. However, as with SOD, there were no significant differences in the levels of soil N supply between plots indicating that, despite invasion by *M. quinquenervia*, overall microbial activity appears to be unaltered (Table 3). This finding may indicate a potential resilience of soil microbial community function to invasion by *M. quinquenervia*.

The extensive and devastating effects of exotic plant invasion on aboveground communities have been well documented (Crooks 2002; D'Antonio and Meyerson 2002; Mack and D'Antonio 1998). However, fewer studies have examined how differences in aboveground plant communities may translate into alteration of belowground systems (Ehrenfeld 2003; Mack et al. 2000; Wardle et al. 2004). Our study reveals that invasion of *M. quinquenervia* caused significant changes in both above- and belowground communities, however, plant mediated changes did not result in significant differences in microbial function. Further testing is needed to determine what factors control microbial activity in these sites in order to predict future implications of *M. quinquenervia* invasion on overall ecosystem function.

With the widespread introduction and invasion of exotic plants there is a vital need for studies that investigate alteration of basic ecosystem structure and function. Our study quantified changes to basic soil parameters following invasion by an exotic, invasive tree. The dearth of studies in this area provides no guidance on long-term implications for the below ground components of this ecosystem.

However, based on the results of this study, we predict that after the invasion of *M. quinquenervia* ecosystem services like nutrient storage will decline with negative consequences for water quality and native community succession.

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